

Amendments to the Specification:

Please replace the paragraph beginning on page 1, line 6 with the following amended paragraph:

This application is a divisional application of U.S. patent application serial no. 09/302,596, filed April 30, 1999, issued as U.S. Patent No. 6,284,725, which is a continuation-in-part of provisional application No. 60/103,498 filed Oct. 8, 1998.

Please replace the paragraph beginning on page 1, line 9 with the following amended paragraph:

This invention relates to metabolic intervention with glucagon-like peptide-1 (GLP-1) ~~GLP-1~~ to therapeutically improve the function of ischemic and reperfused tissue.

Please replace the paragraph beginning on page 3, line 36 with the following amended paragraph:

With respect to the treatment of ischemia coincident with myocardial infarction (MI) ~~MI~~ patients, common therapies now used are to employ thrombolytics such as streptokinase and t-PA and angioplasty. U.S. Pat. No. 4,976,959 discloses the administration of t-PA and SOD to inhibit tissue damage during reperfusion and/or percutaneous transluminal coronary angioplasty coincident with ischemia to restore regional blood flow. Thus, an increasing number of patients are being exposed to the likelihood of reperfusion injury and its effects, particularly cardiac patients.

Please replace the paragraph beginning on page 4, line 29 with the following amended paragraph:

The use of metabolic intervention as a therapy specifically during acute myocardial infarction is well established, although not without controversy. There is abundant experimental and clinical evidence to support the use of a glucose-insulin-potassium (GIK) infusion--the primary form of metabolic intervention--after acute MI, particularly following the success of the Swedish

Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) DIGAMI study (Malmberg, K, and DIGAMI Study Group (1997) Prospective randomized study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. Brit. Med. J. 314, 1512-1515). The DIGAMI study emphasized the efficacy of a glucose-insulin infusion for acute MI in diabetic patients, but this type of therapy has never been suggested or used for reperfusion.

Please replace the paragraph beginning on page 8, line 4 with the following amended paragraph:

From the above discussion it is evident that the dual action of glucose-insulin--enhanced glucose uptake and metabolism, and reduced FFA levels--has substantial therapeutic potential in reperfusion. Some have expressed a concern that during profound, essentially zero-flow ischemia, glycolytic end products, namely lactate, will accumulate due to inadequate "wash-out". Lactate-accumulation, in turn, leads to high intracellular proton concentrations, and failure to reoxidize Nicotinamide Adenine Dinucleotide (NADH) NADH; high $[H^+]$ and NADH/NAD⁺ ratios inhibit productive glycolysis. Under these circumstances, glucose can be toxic to cells, because ATP is actually consumed in the production of fructose-1,6-bisphosphate, and high $[H^+]$ can aggravate myocyte necrosis (Neely, J R, and Morgan, H E (1974) Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Ann. Rev. Physiol. 36, 413-459). However, these concerns have not been borne out by the weight of experimental and clinical data, which indicate that glucose-insulin produces beneficial results. While not wishing to be bound by theory, the likely explanation for this is that in humans, acute spontaneous ischemia is not a condition of zero-flow ischemia, but instead represents a region of low-flow ischemia in which residual perfusion is adequate for substrate delivery and lactate washout. This realization has now provided a powerful physiological logic for the use of metabolic therapy in ischemia-reperfusion.

Please replace the paragraph beginning on page 9, line 20 with the following amended paragraph:

In our previous application (Serial No. 60/103,498), of which this is a continuation-in-part, we reviewed the disadvantages of glucose-insulin infusions and the advantages of substituting these with a GLP-1 infusion, which is safer than insulin. In summary, glucose-insulin-potassium (GIK) ~~GIK~~ infusions carry significant risks of both hypoglycemia and hyperglycemia, and are technically demanding and staff-intensive. The dangers of hypoglycemia are obvious.

Please replace the paragraph beginning on page 13, line 18 with the following amended paragraph:

Mammalian GLP peptides and glucagon are encoded by the same gene. In the ileum the phenotype is processed into two major classes of GLP peptide hormones, namely GLP-1 and GLP-2. There are four GLP-1 related peptides known which are processed from the phenotypic peptides. GLP-1 (1-37) has the sequence His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly (SEQ ID NO:1) (~~SEQ ID NO:1~~). GLP-1 (1-37) is amidated by post-translational processing to yield GLP-1 (1-36) NH₂ which has the sequence His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg (NH₂) (SEQ ID NO:2) (~~SEQ ID NO:2~~); or is enzymatically processed to yield GLP-1 (7-37) which has the sequence His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly (SEQ ID NO:3) (~~SEQ ID NO:3~~). GLP-1 (7-37) can also be amidated to yield GLP-1 (7-36) amide which is the natural form of the GLP-1 molecule, and which has the sequence His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln ~~[[Gln]]~~ Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg (NH₂) (SEQ ID NO:4) (~~SEQ ID NO:4~~) and in the natural form of the GLP-1 molecule.

Please replace the paragraph beginning on page 14, line 3 with the following amended paragraph:

Intestinal L cells secrete GLP-1 (7-37) (SEQ ID NO:3) (~~SEQ. ID NO:3~~) and GLP-1 (7-36) NH₂ (SEQ ID NO:4) (~~SEQ. ID NO:4~~) in a ratio of 1 to 5, respectively. These truncated forms of GLP-1 have short half-lives in situ, i.e., less than 10 minutes, and are inactivated by an aminodipeptidase IV to yield Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly (SEQ ID NO:5) (~~SEQ. ID NO:5~~); and Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg (NH₂) (SEQ ID NO:6) (~~SEQ. ID NO:6~~), respectively. The peptides Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly (SEQ ID NO:5) (~~SEQ. ID NO:5~~) and Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg (NH₂) (SEQ ID NO:6) (~~SEQ. ID NO:6~~), have been speculated to affect hepatic glucose production, but do not stimulate the production or release of insulin from the pancreas.

Please replace the paragraph beginning on page 14, line 20 with the following amended paragraph:

There are six peptides in Gila monster venoms that are homologous to GLP-1. Their sequences are compared to the sequence of GLP-1 in Table 1.

TABLE 1

- a. H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R N H₂
- b. H S D G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S N H₂
- c. D L S K Q M E E E A V R L E T E W L K N G G P S S G A P P P S N H₂
- d. H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S N H₂
- e. H S D A T F T A E Y S K L L A K L A L Q K Y L E S I L G S S T S P R P P S S

f. HSDATFTA EY SKLLAKLALQKYLE SILGSSTSPRPPS
g. HSDAIFTEEY SKLLAKLALQKYLASILGSRTSPPPNH₂
h. HSDAIFTQQY SKLLAKLALQKYLASILGSRTSPPPNH₂

a = GLP-1 (SEQ ID NO:4) (~~SEQ. ID NO:4~~).

b = Exendin 3 (SEQ ID NO:7) (~~SEQ. ID NO:7~~).

c = Exendin 4 (9-39 (NH₂)) (SEQ ID NO:8) (~~SEQ. ID NO:8~~).

d = Exendin 4 (SEQ ID NO:9) (~~SEQ. ID NO:9~~).

e = Helospectin I (SEQ ID NO:10) (~~SEQ. ID NO:10~~).

f = Helospectin II (SEQ ID NO:11) (~~SEQ. ID NO:11~~).

g = Helodermin (SEQ ID NO:12) (~~SEQ. ID NO:12~~).

h = Q⁸, Q⁹ Helodermin (SEQ ID NO:13) (~~SEQ. ID No:13~~).